FastPure Cell/Tissue Total RNA Isolation Kit V2

RC112

Instruction for Use

Version 22.2

Vazyme

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01/Product Description

The FastPure Cell/Tissue Total RNA Isolation Kit V2 provides a rapid method for extracting total RNA from animal tissues and cells. The kit is based on silica gel membrane purification technology and does not require β-mercaptoethanol, phenol/chloroform, or any other toxic reagent during the extraction process. It takes only 6 min to extract high-quality RNA. The kit contains FastPure gDNA-Filter Columns III which can effectively remove impurities and gDNA. FastPure RNA Columns III can efficiently bind RNA with an optimized buffer to obtain high-purity total RNA. The isolated RNA has little gDNA residues and no proteins or other impurities contamination. It can be used for RT-PCR, Real-Time PCR, Microarrarys and other downstream experiments.

02/Components

Components	RC112-01 (50 rxns)
Buffer RL	35 ml
Buffer RW1	45 ml
Buffer RW2	20 ml
RNase-free ddH_2O	10 ml
FastPure gDNA-Filter Columns III (each in a 2 ml Collection Tube)	50
FastPure RNA Columns III (each in a 2 ml Collection Tube)	50
RNase-free Collection Tubes 1.5 ml	50

Buffer RL: Provide the environment required for animal tissue and cell lysis.
Buffer RW1: Remove impurities such as proteins and DNA.
Buffer RW2: Remove salt ion residues.
RNase-free ddH_2O: Elute total RNA.
FastPure gDNA-Filter Columns III: Adsorb DNA and remove impurities in the lysate.
FastPure RNA Columns III: Adsorb RNA specifically.
Collection Tubes 2 ml: Collect filtrate.
RNase-free Collection Tubes 1.5 ml: Collect RNA.

03/Storage

Store at 15 ~ 25℃ and transport at room temperature.

04/Applications

Animal tissues (10 - 20 mg)
Cultured cells ($<5 \times 10^6$)

05/Self-prepared Materials

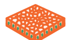
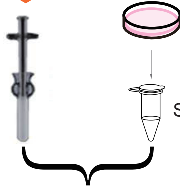
Absolute ethanol, 50% ethanol, 1.5 ml RNase-free centrifuge tubes, RNase-free pipette tips, etc.

06/Notes

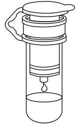
For research use only. Not for use in diagnostic procedures.

1. Please add 80 ml absolute ethanol to Buffer RW2 according to the label, mark the bottle body and cap and mix well before use.
2. Check whether there is crystal precipitation in Buffer RL and Buffer RW1 before use. If there is crystal precipitation, it can be placed at 37℃ to dissolve the precipitation, and then mixed thoroughly before use.
3. The amount of sample processing should be controlled at 10 - 20 mg tissue or <5 × 10^6 cells. For tissues rich in DNA/RNA such as liver, spleen, kidney, etc., do not input more than 10 mg. Otherwise, it will cause gDNA residues or low yield, and may also lead to clogging of FastPure gDNA-Filter Columns III.
4. Please use fresh samples. If the samples cannot be extracted in time, immediately place the samples in liquid nitrogen for quick freezing, store them at -85 ~ -65℃, and avoid repeated freezing and thawing. Alternatively, the samples can be homogenized immediately in Buffer RL and stored at -85 ~ -65℃.
5. Incomplete disruption of the sample will affect RNA yield and easily cause column blockage. Please try to control the temperature during homogenization to prevent RNA degradation caused by high temperature.
6. For extraction of RNA from liver tissue, please read 08/Experiment Process carefully.
7. When using this kit, please wear laboratory coat, disposable latex gloves, disposable mask and use RNase-free consumables to prevent RNase contamination.
8. All procedures must be carried out at room temperature (15 ~ 25℃).

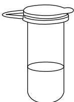
07/Mechanism & Workflow

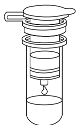
Sample lysis: Add 500 μl Buffer RL



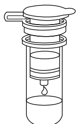
gDNA removal: Transfer the lysate to a FastPure gDNA-Filter Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Collect the filtrate**.



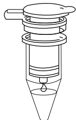
Adjust the binding environment: Add absolute ethanol (0.5 × the volume of filtrate) to the filtrate and mix thoroughly.



RNA adsorption: Transfer the mixture into FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. **Discard the filtrate**.



Impurities removal: Add 700 μl Buffer RW1 to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.
Add 700 μl Buffer RW2 (with absolute ethanol added) to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.
Add 500 μl Buffer RW2 (with absolute ethanol added) to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the collection tube.



RNA elution: Add 50 - 200 μl RNase-free ddH_2O to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 1 min.

08/Experiment Process

Please read this instruction carefully before starting the experiment.

◇ Please add 80 ml absolute ethanol to Buffer RW2 according to the label, mark the bottle body and cap and mix well before use.

◇ For liver tissue samples, prepare 50% ethanol with RNase-free ddH_2O in advance.

◇ Check whether there is crystal precipitation in Buffer RL and Buffer RW1 before use. If there is crystal precipitation, it can be placed at 37℃ to dissolve the precipitation, and then mixed thoroughly before use.

08-1/Sample Processing

Animal tissue

◇ Homogenization: Add 500 μl (350 μl for liver tissue) Buffer RL to every 10 - 20 mg of fresh tissue. Using a glass or electric homogenizer to homogenize until there are no visible tissue pieces.

▲ Perform the homogenization on ice to prevent RNA degradation caused by transient increase in local temperature.

◇ Liquid nitrogen grinding: After grinding the sample using liquid nitrogen, immediately transfer to Buffer RL, adding 500 μl (350 μl for liver tissue) Buffer RL per 10 - 20 mg of pulverized sample. Vortex until there are no visible clumps of powder.

▲ After homogenization or liquid nitrogen grinding, samples can be stored at -85 ~ -65℃.

Cultured cells

◇ Adherent cells: Buffer RL can be used for digestion and lysis directly in the cell culture dish after cell culture supernatant is removed. Or collect trypsin-digested cells by centrifugation and add 500 μl Buffer RL to every <5 × 10^6 cells. Vortex until there are no visible clumps of cells.

◇ Suspending cells: Collect the cells directly by centrifugation. Add 500 μl Buffer RL to every <5 × 10^6 cells and vortex until there are no visible clumps of cells.

▲ After cell lysis, samples can be stored at -85 ~ -65℃.

08-2/RNA Extraction

Perform the following steps in RNase-free environment.

1. Transfer the lysate to FastPure gDNA-Filter Columns III (FastPure gDNA-Filter Columns III had been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the FastPure gDNA-Filter Columns III and **collect the filtrate**.

2. Add absolute ethanol (0.5 × the volume of filtrate) to the filtrate and mix thoroughly. For liver tissue, add 50% ethanol (1 × the volume of filtrate).

   ▲ It is normal for the solution to turn turbid or to contain flocculent precipitate after adding ethanol. Mix the solution well by vortexing and use it directly in the next step.

3. Transfer the mixture from Step 2 to FastPure RNA Columns III (FastPure RNA Columns III had been put into the collection tube) and centrifuge at 12,000 rpm (13,400 × g) for 30 sec and discard the filtrate.
4. Add 700 μl Buffer RW1 to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.
5. Add 700 μl Buffer RW2 (check if absolute ethanol has been added in advance!) to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the filtrate.
6. Add 500 μl Buffer RW2 (check if absolute ethanol has been added in advance!) to the FastPure RNA Columns III and centrifuge at 12,000 rpm (13,400 × g) for 2 min. Carefully remove the adsorption column from the collection tube to avoid contacting with the filtrate to prevent contamination.
7. (Optional) If the adsorption column has residual liquid or contacts with the filtrate, discard the filtrate and place the FastPure RNA Columns III back into the collection tube. Centrifuge at 12,000 rpm (13,400 × g) for 1 min to prevent ethanol contamination.
8. Transfer the adsorption columns to new RNase-free Collection Tubes 1.5 ml carefully. Add 50 - 200 μl of RNase-free ddH_2O to the center of the adsorption column without touching the membrane and incubate at room temperature for 1 min. Centrifuge at 12,000 rpm (13,400 × g) for 1 min to elute the RNA.
   ▲ In order to improve the yield, RNase-free ddH_2O could be preheated at 65℃ in advance, and after adding the RNase-free ddH_2O to the membrane, incubate at room temperature for 2 - 5 min. Or secondary elution the RNA after centrifugation.
9. The total RNA extracted can be directly used for downstream experiments or stored at -85 ~ -65℃.

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09/FAQ & Troubleshooting

FAQ	Reasons	Solutions
Clogged adsorption column	1. Too much starting material	Reduce the amount of sample added. Do not add more than 10 mg tissues rich in DNA/RNA, such as liver, spleen, and kidney.
	2. The sample is rich in muscle fibers	Process the sample by grinding it with liquid nitrogen. Increase the intensity of grinding for tissues rich in muscle fiber, such as muscular, cardiac, and skin tissue.
	3. Insufficient grinding or homogenization of tissues	Centrifuge the lysed sample at 12,000 rpm (13,400 × g) for 5 min, and then collect the supernatant for subsequent extraction.
Low or no recovery of RNA	1. Too little amount of sample	Increase the amount of sample added. Do not exceed 20 mg of tissues or 5 × 10^6 cells.
	2. Improper storage of sample	Endogenous RNase has degraded the RNA. Use fresh sample or sample stored at -85 ~ -65℃ that have never been thawed.
	3. Insufficient grinding or homogenization of tissue	Increase the volume of lysis buffer and lysis time.
	4. Improper elution	Add RNase-free ddH_2O to the center of the membrane and reduce the elution volume as appropriate. Preheat the RNase-free ddH_2O to 65℃, and prolong the incubation time or perform secondary elution.
RNA degraded	1.The sample is not stored promptly	Use fresh sample or sample stored at -85 ~ -65℃ that have never been thawed.
	2.Repeated freezing and thawing of sample	Avoid repeated freezing and thawing and store in aliquots.
	3.Contamination from electrophoresis or the environment	Before the electrophoresis, soak the electrophoresis tank in 3% hydrogen peroxide for 20 min and rinse it with RNase-free ddH_2O. Use RNase-free ddH_2O to prepare electrophoresis buffer to ensure the RNase-free extraction environment.
Inhibition of downstream reaction or low purity	1.Salt ion residues	Ensure that washing with Buffer RW2 is performed twice. Additionally, add Buffer RW2 to the sides of the adsorption column or close the lid of the column and invert 2 to 3 times after adding Buffer RW2, which can help to completely wash away any salts on the sides of the column.
	2.Ethanol residues	Perform Step 7.
gDNA contamination	1.Excessive amount of sample	The DNA/RNA content of different samples varies greatly, do not exceed 20 mg of tissues or 5 × 10^6 cells. Do not use more than 10 mg of tissues rich in DNA, such as liver, spleen, and kidney. Residual gDNA can be further removed by digestion using DNase. For details, please contact us (web: www.vazyme.com).

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